

etiologic agent was found, so it was considered that AA was secondary to HIV. Treatment was initiated with D4T (Stavudine), 3TC (Lamivudine) and Efavirenz achieving undetectable viral load and increasing CD4 count. Patient also received Erythropoietin (EPO) and granulocyte-colony stimulating factors (G-CSF) showing an increased number of white blood cells (WBC) but continued with high transfusional requirement. We had to stop treatment in October because of liver failure and lactic acidosis. In November we changed Stavudine for Tenofovir and reiniciated treatment. Viral load was always undetectable and CD4 count was > 500 cells/ul. However megakaryocytopoiesis and erythropoiesis did not respond, requiring many transfusions. Coombs Direct Test (CDT) and Coombs Indirect Test (CIT) were positive. He showed an immunohaematologic profile with 1 autoantibody (Anti-e) and 3 alloantibodies (Anti-Jka, Anti-Lua, Anti-Cw).

As he had an identical twin, he was submitted to a syngeneic BMT. Although he was heavily transfused (421 Units), we did not want to increase immunosuppression so as not to have viral reactivation. The conditioning regimen consisted of Cy 50 mg/kg/qd x 4, and in order to lower the risk of engraftment failure, peripheral blood stem cells were used to maximize the number of donor cells infused ( $11 \times 10^6$  CD34+ cells/kg). At the transplantation the patient was in high-risk. The antiretroviral treatment was not discontinued. No graft versus host disease (GVHD) prophylaxis was needed. Neutrophils and platelets engrafted at day +11.

After 10 months of transplantation he continues in complete haematologic remission. Serum antibodies, CDT and CIT are negative. Viral load remains undetectable (b-DNA).

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### ABSOLUTE CHIMERISM AS A TOOL IN MONITORING IMMINENT AND MANIFEST GRAFT REJECTION AFTER HEMATOPOIETIC CELL TRANSPLANTATION WITH NONMYELOABLATIVE CONDITIONING

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Graft rejection after hematopoietic cell transplantation (HCT) with nonmyeloablative conditioning is a rare event and a serious clinical problem. Manifest (N=8) and imminent rejection (N=4) in a cohort of 98 consecutive patients with hematological malignancies were analyzed. The patients were conditioned with fludarabine 30 mg/m<sup>2</sup> and 2 Gy of total body irradiation and transplanted with peripheral blood stem cells. Intervention aiming at reversing imminent rejection with donor lymphocyte infusion (DLI) alone or preceded by immunosuppression with pentostatin was attempted with highly variable results. Chimerism analysis is the standard method to monitor engraftment and rejection. In the present report we have evaluated the product of absolute T cell counts and chimerism, which we have termed absolute chimerism, for monitoring patients with manifest or imminent rejection. The results suggest that recipient T cell counts > donor T cell counts and increasing recipient T cells post-transplant are risk factors for rejection. Peaks of absolute recipient CD4+ and/or CD8+ T cell counts were seen in relation to rejection and peaks of donor CD4+ and/or CD8+ T cells were seen in connection with acute graft-versus-host disease. Furthermore absolute chimerism plots in some cases clearly indicate the time-interval where disappearance of recipient T cells takes place. These findings may be of importance for understanding the cellular mechanisms underlying alloreactivity. Absolute chimerism plots point to a variable immunosuppressive effect of pentostatin on recipient T cells as explanation for pentostatin/DLI-failure in reversing rejection. We conclude that absolute chimerism plots can contribute significant new information of value for routine monitoring of patients with mixed chimerism as well as for research purposes. Following rejection patients are at risk of dying from infections and progression/relapse of their malignancy. Retransplantation is feasible and well tolerated after HCT with nonmyeloablative conditioning. In patients with imminent graft rejection retransplantation is an attractive alternative to DLI or immunosuppression/DLI.

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### AN IMPROVED METHOD FOR ENGRAFTMENT MONITORING USING REAL-TIME PCR

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The commonly used engraftment monitoring method of short tandem repeat (STR) amplification following allogeneic hematopoietic stem cell transplantation (HSCT) is hampered by several shortcomings affecting sensitivity, accuracy, reproducibility, and results interpretation. In an effort to provide a better solution, we have developed a truly quantitative, real-time PCR method. Real-time PCR technology is ideally suited for this application because it is highly sensitive and quantitatively much more accurate and precise than STR analysis. It is not affected by problems inherent to STR amplification and analysis such as plateau bias, preferential allele amplification, and stutter artifacts. With our method, genomic mixtures are easily resolved, and analysis is straightforward and automatable. Success and maintenance of allogeneic HSCT may be enhanced with such an improved engraftment monitoring method.

We have developed a panel of non-repetitive polymorphic markers, with representatives on all chromosomes, useful for identifying differences between recipient and donor pairs. Our statistical analyses given allele frequencies in multiple human populations demonstrate that with relatively high minor allele frequencies, a limited panel of markers can be assembled which would have high probability of providing informative markers that could distinguish HLA-matched individuals. The testing process first requires that the donor and recipient genomic DNA be screened in order to identify informative markers between the two genomes. Once defined, these markers are used post-transplant to quantify the relative percentage of recipient genetic material in the donor background. Compared to the current STR assays, our method shows a greater than 100-fold increase in sensitivity with excellent accuracy and precision at concentrations as low as 0.01% recipient in a background of 99.99% donor DNA. Given the reproducibility of results and ease of data analysis, this approach provides the means for standardization within a testing lab as well as between testing centers, which cannot be accomplished with homebrew and off-label methods such as STR analysis. From a clinical perspective, improved sensitivity, accuracy, and precision provide the potential to detect disease relapse or transplant rejection at a much earlier stage. This information may be useful for improving decisions related to maintenance treatments such as GVHD prophylaxis and/or donor lymphocyte infusion.

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### CHARACTERISTICS OF TWO CONDITIONING REGIMENS CYCLOPHOSPHAMIDE PLUS ANTITHYMOCYTE GLOBULIN VERSUS CYCLOPHOSPHAMIDE PLUS BUSULFAN IN ALLOGENEIC STEM CELL TRANSPLANTATION FOR SEVERE APLASTIC ANEMIA

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We report a retrospective study of the clinical features and outcome for 32 allogeneic stem cell transplants (AlloSCT) from HLA-matched sibling donors in severe aplastic anemia conditioned with 200 mg/kg of cyclophosphamide plus 90 mg/kg of horse antithymocyte globulin (ATG) in 17 patients and 120 mg/kg of cyclophosphamide plus 12 mg/kg of busulfan (Bu) in 15 patients, at Hospital São Paulo and Hospital Santa Marcelina, from November 1993 to August 2003. Considering high cost of ATG and the difficulty to obtain it for the majority of our centers, the objective was to compare clinical variables as age, sex, engraftment, interval of time from diagnosis to AlloSCT, number infused of total nuclear cells, previous transfusions, occurrence of acute and chronic GVHD, infections, acute and late graft rejection and overall survival, between these two conditioning regimens. We analyzed the long-term hematopoietic chimerism by FISH, using a